

# Assessing the Mating Status of Female Codling Moth (Lepidoptera:Tortricidae) in Orchards Treated with Sex Pheromone Using Traps Baited with Ethyl (*E*, *Z*)-2,4-Decadienoate

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**ABSTRACT** Whether sticky traps baited with ethyl (*E*, *Z*)-2,4-decadienoate (pear ester) can be used to accurately assess the mating status of female codling moth, *Cydia pomonella* L., was evaluated in a series of tests. The proportion female moths caught in these traps that were virgin was compared with catches using interception and light traps in two separate studies. The mean proportion of virgin female codling moths caught in pear ester-baited traps was significantly lower than caught with either interception or light traps. Results were similar in both untreated and sex pheromone-treated orchards. Cohorts of virgin and mated female codling moths were flown separately to a pear ester-baited trap placed overnight in a flight tunnel. The recapture rate of virgin moths was significantly lower than for mated moths. The relation of the mean proportion of females mated versus the density of female moths caught in pear ester-baited traps over the entire season was examined by grouping data from 180 traps into eight density classes from 1 to >20 female moths per trap per season. A significantly higher mean proportion of virgin females were caught in the lowest density class (one moth per trap) than in all other classes. Traps catching two female moths per season had a significantly higher proportion of virgin moths than traps catching 4–5, 9–15, and 16–20 moths. No significant differences in the proportion of virgin moths occurred among traps grouped into classes from 3 to >20 female moths caught per season. These data are consistent with action thresholds previously established for cumulative catch of female moths in pear ester-baited traps (at least one moth) and support the use of this kairomone to assess the potential efficacy of sex pheromone programs.

**KEY WORDS** *Cydia pomonella*, kairomone, monitoring, pear ester

The major sex pheromone component of codling moth, *Cydia pomonella* L. (Lepidoptera: Tortricidae), (*E*, *E*)-8–10-dodecadien-1-ol (codlemone), has been widely used to manage this key pest of apple, pear, and walnut through mating disruption (Vickers and Rothschild 1991, Cardé and Minks 1995). A variety of codlemone-based technologies have been developed, such as hand-applied dispensers (Howell et al. 1992), widely dispersed aerosol emitters (Shorey and Gerber 1996) or clusters of dispensers (Knight 2004a), microencapsulated sprayables (Knight and Larsen 2004), and broadcast dispersion of microfibers (Knight 2003). The effectiveness of these technologies to manage codling moth is presumed to be caused by the disruption of mating and the concurrent reductions in the reproductive potential of this pest (Vickers and Rothschild 1991). However, significant reductions in moth fecundity can also occur because of a delay in mating, and this has been proposed as an important alternate factor impacting the success of these technologies (Vickers 1996, Knight 1997).

Light traps (Howell and Britt 1994) and a passive interception trap (Knight 2000) have been used to assess levels of mating of codling moth populations in orchards treated with sex pheromone. The mating status of the female moths caught with an oil-coated interception trap has been shown to accurately reflect the status of field populations (Knight 2000). The proportion of female codling moths caught on interception traps that were virgin was 0.40 and 0.12 in sex pheromone-treated and -untreated orchards in 1995–1996 (Knight 2000). Light traps are known to catch reproductively younger moths than bait traps (Geier 1960); however, whether they are biased in capturing either mated or virgin codling moths is unclear. The mean proportion of female codling moths that were virgin caught in light traps placed in 19 sex pheromone-treated orchards during 1994 was 0.37 (Howell and Britt 1994). Unfortunately, they did not collect similar data from untreated orchards. Both light and interception traps have not been widely adopted to monitor codling moth or to assess mating disruption, perhaps because of their relatively high operation costs.

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The recent identification of ethyl (*E*, *Z*)-2,4-decadienoate (pear ester) as a bisexual attractant for codling moth has provided a simple and inexpensive tool to monitor female codling moth populations (Light et al. 2001). The use of pear ester to effectively monitor the mating status of female codling moth has been highlighted as one of its most important uses (Light et al. 2001). The proportion of female codling moths caught in pear ester-baited traps placed in both walnut (Light et al. 2001) and apple orchards (Knight and Light 2005a) treated with sex pheromones that were virgin has been surprisingly low. During most weeks of the season, this proportion was  $<0.20$ . However, pear ester-baited traps did detect seasonal variability in mating, with higher proportions of virgin females (0.20–0.50) trapped at the start of the spring moth flight, between summer flights, and at the end of the season (Light et al. 2001, Knight and Light 2005a).

Additional information is needed to assess whether pear ester-baited traps provide an unbiased assessment of female codling moth mating in sex pheromone-treated orchards. Differences observed over a nearly 10-yr time span in the proportion of female codling moths mated in sex pheromone-treated orchards may be caused by differences in the trapping methods, the effects of supplemental pest management controls, changes in the use of sex pheromone technologies, or changes in their effectiveness. Clearly, the development of new behavior-modifying management approaches and further optimization of the use of sex pheromones would benefit from an ability to accurately assess their direct impact on mating.

Herein, results are reported from three studies that address the use of pear ester-baited traps to measure the mating status of female codling moth populations. First, comparisons were made of the mating status of female codling moth caught in pear ester-baited sticky traps, oil-coated interception traps, and UV light traps. Second, the recapture rates of both virgin and mated female codling moths to a pear ester-baited trap placed overnight in a flight tunnel was evaluated. Third, the proportion of virgin females was assessed in a large number of orchards treated with sex pheromones, and these data were analyzed as a function of female moth density.

## Materials and Methods

**Proportion of Female Moths Mated as Caught in Different Trap Types.** Studies were conducted in eight apple orchards to evaluate the proportion of females mated that were caught in sticky traps baited with pear ester versus oil-covered interception traps during 2000–2002. Orchards were situated near Parker, Zillah, and Moxee, WA. The two Parker orchards were monitored from 2001 to 2002. The two Zillah orchards were monitored from 2000 to 2002. Neither the Parker or Zillah orchards were treated with sex pheromone dispensers or with insecticides after petal fall each year. Four or five Moxee orchards were monitored each year from 2000 to 2002. All Moxee orchards were treated with 1,000 Isomate-C

PLUS dispensers (Pacific Biocontrol, Vancouver, WA) per hectare. The manufacturer's label specifies that dispensers were loaded with 182.3 mg of a 60:33:7 blend of (*E*, *E*)-8–10-dodecadien-1-ol, dodecan-1-ol, and tetradecan-1-ol. All orchards were mixed plantings of 'Delicious' and 'Golden Delicious' and planted at densities of 500–600 trees/ha. Mean tree heights averaged 4.0–4.5 m among orchards.

Orchards were monitored with both a delta-shaped plastic trap with a removable sticky liner and baited with a pear ester lure (Pherocon CM-DA; Trécé, Adair, OK) and groups of nine interception traps (one trap placed per tree on a 3 by 3 grid of trees). Trapping sites were  $>30$  m from the border of each orchard and separated by 50–150 m depending on the size of the orchard. Interception traps (0.10 m<sup>2</sup>) were cut from 1.26 by 30.4-m rolls of 0.25-mm clear plastic (Yakima Plastics, Yakima, WA). A 20-cm piece of plastic strapping was threaded through a hole punched in the center of the top edge of each trap and was fastened to a plastic clip (Suterra, Bend, OR) for attachment to tree branches. Interception traps were coated with a thin layer of oil (STP Oil Treatment; STP, Ft. Lauderdale, FL) just before their placement in the field. Traps were clipped on branches in the upper third of the canopy using a 4.0-m telescoping pole. Delta-shaped traps were attached to PVC poles and also hung in the upper third of the canopy. Traps were placed in orchards in mid-April each year and checked daily until 19 May and 23 May in 2000 and 2001, respectively, and every 3–4 d after those dates and during 2002 until early September. Sticky liners in delta traps were changed frequently—at least every 4 wks during the season. Lures were replaced once after 9–10 wk each season. Moths were removed from traps and sexed in the laboratory. Female moths were dissected to determine their mating status (presence or absence of one or more spermatophores in the bursa copulatrix). Data are summarized for each of the two moth flights using data before and after 1 July.

The proportion of mated female codling moths caught in pear ester-baited traps versus light traps was compared in five Moxee orchards treated with 1,000 Isomate-C PLUS dispensers/ha during 2003. One pear ester-baited delta trap and light trap were placed at  $>50$  m from the border and 100–150 m apart in orchards on 9 June and monitored weekly until 5 September. Light traps were constructed of galvanized metal and illuminated with a 6-W F6T5/BL T5 fluorescent blacklight bulb (Topbulb.com, East Chicago, IN), and powered by a 12-V solar charged battery. Lights were automatically switched on each evening from 2100 to 2400 hours by a time clock. A dichlovos-impregnated (18.6% AI) plastic strip (Hot Shot No Pest Strip; United Industries, St. Louis, MO) was placed in the bottom of each light trap to kill moths. All traps were checked weekly, moths were removed and sexed, and females were dissected to determine their mating status.

**Flight Tunnel Assays.** Tests were conducted to evaluate the effect of female moth's mating status on their recapture in a pear ester-baited trap placed overnight

in a flight tunnel. The flight tunnel was constructed from 6-mm acrylic sheeting (1.66 m long, 0.57 m wide, and 0.57 m high). A 12-V DC blower was used to pull air from the room (maintained at 22–24°C and 50–60% RH) into a plenum, through a charcoal filter, and through a series of screens before passing into the tunnel. Air flow through the tunnel was maintained at 0.25 m/s. Exhaust was expelled to the outside of the building. Red lights installed above the tunnel provided enough light (4.3 lux) to make behavioral observations.

Mature larvae from a laboratory colony inside corrugated cardboard were placed individually in vials and kept at 25°C until adult eclosion. Moths were collected daily, sexed, and stored in vials at 5°C until used in experiments. All female moths were transferred to 416-ml plastic cups in a room maintained at 25°C and >45% RH for 1 d before testing. Female moths presumed to be mated had been placed in cups with a virgin 1-d-old male moth before the test. Cohorts of five virgin or presumed-mated female moths were released each day in the flight tunnel at 1400 hours. Presumed-mated and virgin moths were flown on alternate days ( $n = 10$  replicates).

A diamond-shaped Pherocon IIB cardboard trap (Trécé) with a sticky interior surface was placed level on a ring stand 0.31 m above the tunnel floor and 0.20 m from the upwind end of the tunnel. A single halobutyl gray septum loaded with 3.0 mg pear ester (>98.0% purity; Trécé) was pinned inside to the middle of the trap. Traps were checked the following morning at 0700 hours, and the presumed-mated females were dissected to determine their actual mating status. The mating status of female moths not caught in the trap was also determined.

**Assessment of Mating with Pear Ester-baited Traps.** Studies were conducted from 2000 to 2002 in 125 orchards situated near Brewster and Yakima, WA. Orchards in Brewster were a mix of apple cultivars: 'Gala', 'Golden Delicious', 'Delicious', and 'Fuji'. Orchards in Yakima were mixed blocks of 'Delicious' and 'Golden Delicious'. All orchards were treated with 500–1,000 Isomate-C PLUS dispenser/ha. Most orchards were also treated with one to three spray applications of the organophosphate insecticides (azinphosmethyl [Micro Flo Co., Memphis, TN] and phosmet [Zeneca Ag Products, Wilmington, DE]) or an insect growth regulator (IG; methoxyfenozide; Dow AgroSciences, Indianapolis, IN).

Delta-shaped traps ( $n = 2$ ) with removable sticky liners and baited with the pear ester Pherocon CM-DA lure were attached to PVC poles and hung in the upper third of the canopy of each orchard. Traps were spaced >100 m apart and placed in orchards from the last week in April to the first week of May during each year and monitored for 18 wk until September. All traps were checked weekly. Sticky liners were changed at least every 4 wk during the season. Lures were replaced once after 9–10 wk each season. All moths were collected from traps and returned to the laboratory. Moths were sexed, and females were dissected to determine their mating status.

**Statistical Analysis.** All proportional data were subjected to Bartlett's correction before angular transformation (Snedecor and Cochran 1967). A paired  $t$ -test was used to compare the proportion of females caught in pear ester-baited versus either interception or light traps that were mated (Analytical Software 2003). Data for each orchard-year were treated as a replicate. A two-sample  $t$ -test was used to analyze the influence of mating status on female moth recapture in a pear ester-baited trap placed in the flight tunnel. Seasonal moth catches per trap for 180 traps were grouped into eight density classes: 1, 2, 3, 4–5, 6–8, 9–15, 16–20, and >20 female moths based on creating classes with nearly equal number of replicates (20–26). The mean proportion of females found mated for traps within each class was analyzed with analysis of variance (ANOVA). Means were separated in significant ANOVAs with Fisher test of least significant difference (LSD;  $P < 0.05$ ).

## Results

**Proportion of Female Moths Mated as Caught in Different Trap Types.** Large numbers of female codling moths were caught in clusters of nine interception traps (86–556), pear ester-baited sticky traps (5–226), and light traps (68–376) during these studies. A significantly lower proportion of female codling moths that were virgin were caught on interception traps than in pear ester-baited sticky traps during both moth flights and over the entire season in orchards monitored from 2000 to 2003 (Table 1). This relationship was consistent for both untreated and sex pheromone-treated orchards, except that there was no difference between the two traps over the entire season in untreated orchards ( $P = 0.06$ ; Table 1). Light traps caught a significantly higher mean proportion of female codling moth that were virgin ( $0.16 \pm 0.01$  [SE]) than pear ester-baited sticky traps ( $0.07 \pm 0.02$ ) placed in five orchards during 2003 ( $t = 4.25$ ,  $df = 4$ ,  $P < 0.05$ ).

**Flight Tunnel Assays.** A significantly greater mean proportion of mated ( $0.28 \pm 0.06$ ) versus virgin ( $0.04 \pm 0.03$ ) female codling moths were caught overnight in a pear ester-baited trap placed in a flight tunnel ( $t = 3.64$ ,  $df = 18$ ,  $P < 0.01$ ). All presumed-mated female moths used in these tests were actually mated, including both the moths caught in the trap and those remaining inside the flight tunnel.

**Assessment of Mating with Pear Ester-baited Traps.** Density class of overall numbers of female codling moths caught was a significant factor affecting the mean proportion of female codling moths that were virgin ( $F = 7.87$ ;  $df = 7, 172$ ;  $P < 0.0001$ ). A significantly higher proportion of virgin moths was caught in traps catching only one female moth per season than in all other density classes (Fig. 1). The mean proportion of virgin females was significantly higher when only two females were caught per trap per season versus traps in density classes of 4–5, 9–15, and 16–20 moths. No differences in the mean proportion of virgin moths

**Table 1.** Proportion of trapped female codling moths that were virgin in either delta-shaped sticky traps baited with a pear ester lure or on unbaited oil-coated plastic interception traps placed in apple orchards treated with sex pheromone dispensers or untreated, 2000–2003

Trap	Mean proportion (SE) of female moths that were virgin		
	First flight	Second flight	Entire season
Sex pheromone-treated orchards, <i>n</i> = 14 orchard/yr			
Pear ester-baited trap	0.15 (0.03)	0.09 (0.03)	0.15 (0.02)
Interception trap	0.29 (0.06)	0.16 (0.03)	0.23 (0.04)
Paired <i>t</i> -test, <i>df</i> = 13	<i>t</i> = 4.10, <i>P</i> < 0.01	<i>t</i> = 3.99, <i>P</i> < 0.01	<i>t</i> = 4.57, <i>P</i> < 0.001
Untreated orchards, <i>n</i> = 10 orchard/yr			
Pear ester-baited trap	0.16 (0.04)	0.12 (0.03)	0.16 (0.02)
Interception trap	0.26 (0.02)	0.18 (0.03)	0.20 (0.02)
Paired <i>t</i> -test, <i>df</i> = 9	<i>t</i> = 3.86, <i>P</i> < 0.01	<i>t</i> = 2.30, <i>P</i> < 0.05	<i>t</i> = 2.17, <i>P</i> = 0.06
All orchards, <i>n</i> = 24 orchard/yr			
Pear ester-baited trap	0.16 (0.02)	0.11 (0.02)	0.15 (0.02)
Interception trap	0.28 (0.03)	0.17 (0.02)	0.22 (0.02)
Paired <i>t</i> -test, <i>df</i> = 23	<i>t</i> = 5.63, <i>P</i> < 0.0001	<i>t</i> = 4.43, <i>P</i> < 0.001	<i>t</i> = 4.82, <i>P</i> < 0.0001

were found for traps grouped into density classes from 3 to >20 female moths per season (Fig. 1).

### Discussion

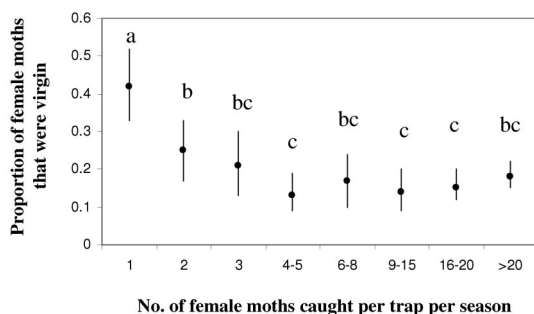
A significant difference in the attractiveness of pear ester-baited traps to mated versus virgin female codling moths was established in both field and laboratory tests. This is interesting in regards to studies reporting that the antennal chemoreceptive responses of mated and virgin codling moth females to pear ester are similar (Avilla et al. 2003, De Cristofaro et al. 2004). Female codling moth antennae respond to a wide range of esters and terpenoid compounds characteristic of apple foliage and fruit at different phenological stages of the host (Bäckman et al. 2001, Bengtsson et al. 2001, Light et al. 2001, Ansebo et al. 2004). Unfortunately, the behavioral responses of mated versus virgin females to these volatiles have not been reported. Upwind flight of mated females has been shown to both apple fruits and collected volatiles (Reed and Landolt 2002), whereas virgin females did

not respond to pear ester during another particular flight tunnel assay (B. Sauphanor, personal communication).

Several direct comparisons of the attraction responses of mated versus virgin codling moth females have been reported. Virgin and mated female codling moths were both stimulated by a mixture of apple volatiles (Yan et al. 1999) and to  $\alpha$ -farnesene (Hern and Dorn 1999). The intensity of responses of mated females was significantly greater than virgin females in both studies. A purported female-specific attractant, butyl hexanoate, was attractive to mated, but not virgin females in olfactometer tests (Hern and Dorn 2004).

The stronger behavioral response of mated than virgin females to fruit volatiles in these studies is not surprising. Mated female codling moths oviposit in close proximity to fruits (Jackson 1979) and must be able to differentiate the chemical signals emitted by both unripe and ripening fruit within a complex and changing mix of host plant volatiles (Bengtsson et al. 2001). Codling moth oviposition is stimulated by various host volatiles (Witzgall et al. 2005), including the pear ester (Knight and Light 2004). The behaviors of virgin female codling moths have not been well studied, except for various courtship activities (Howell 1991). The potential influences of host volatiles on virgin female codling moth behavior have not been reported.

Identification of pear ester as a kairomone for adult codling moth has created several new approaches to improve both the monitoring and management of this important fruit and nut pest (Light et al. 2001). These include predicting the onset of egg hatch (Knight and Light 2005b), developing action thresholds (Knight and Light 2005c), testing microencapsulated formulations of pear ester alone and in combination with codlemone for mating disruption (Light et al. 2005), and the use of pear ester-baited killing stations (Knight et al. 2002). However, determining the mating status of captured female moths has not been widely adopted (Ioriatti et al. 2003, Thwaite et al. 2004, Trimble and El-Sayed 2005).



**Fig. 1.** Back-transformed mean proportions ( $\pm$ back-transformed 95% CL) of female moths caught in pear ester-baited traps that were virgin as a function of the total number of female moths caught per trap over the entire season. Data from 180 traps were grouped into eight density classes, and ANOVA was conducted with angular transformed means. Means with different lowercase letters were significantly different, LSD test, *P* < 0.05.



However, when conducted, distinction of female mating status did not improve the prediction of the start of egg hatch (Knight and Light 2005b). Here, the numbers of female codling moths caught per trap over the entire season was a significant, although a weak indicator of their mating status.

The significantly higher mean proportion of virgin female codling moths at moth densities of one to two moths per trap per season overlaps the action thresholds based on moth catch previously established for sex pheromone-treated orchards (Knight and Light 2005c). Orchard plots (1.0 ha) surrounding pear ester-baited traps catching at least one female moth early in the season or at least two female moths before 1 July had detectable levels of fruit injury at harvest. This close agreement suggests that seasonal female moth catch can be used as an indicator of both mating success and potential fruit injury within an orchard block treated with sex pheromone. However, the bias of the pear ester lure for mated female moths and its lack of sensitivity at densities of  $>1$  female moth per trap per season also suggests that dissections of female moths are probably not useful or necessary. Nevertheless, similar data sets of mating success as a function of female moth density captured in traps should be gathered to evaluate other sex pheromone-based technologies, such as aerosol puffers (Shorey and Gerber 1996) or microencapsulated sprayables (Knight and Larsen 2004), as well as the influences of the several types and densities of hand-applied dispensers currently used for codling moth (Doerr et al. 2004).

A nearly universal tenet in the use of sex pheromones for management of lepidopteran pests is that these technologies will work better against low versus higher moth population densities (Cardé and Minks 1995). It is generally presumed that the rate of mating is low within treated orchards and that the probability of mating is a function of moth density, especially if false-trail following is an important mechanism of mating disruption (Sanders 1997). A history of successful management of codling moth with Isomate C dispensers (Thomson et al. 2001), despite minimal reductions in the mating success of females, suggests that other mechanisms that can reduce population growth may be important, such as delay of mating (Vickers 1996, Knight 1997) or reductions in female multiple mating (Fadamiro and Baker 1999). Both a delay in the onset of egg hatch (Knight 2004b) and a reduction in the occurrence of multiple mating (Light et al. 2005) have recently been documented, although their potential impacts on the seasonal population dynamics of codling moth in these orchards has not been assessed.

Finally, it is interesting that the proportion of virgin female moths caught with the interception and light traps reported here is much lower than data collected nearly 10 yr ago. Howell and Britt (1994) placed light traps in 19 orchards in the Yakima Valley all treated with 1,000 Isomate-C dispensers/ha in 1994. Traps caught from 1 to 2,688 females per season; however, the proportion of virgin females was fairly consistent across orchards (mean =  $0.37 \pm 0.04$ ). Knight (2000) monitored codling moth on oil-coated interception

traps in 1995–1996 in two of the Moxee orchards included in this study and in one untreated site. Both orchards were treated with 1,000 Isomate-C PLUS dispensers/ha and the mean proportion of female moths trapped that were virgin was  $0.40 \pm 0.06$  compared with  $0.12 \pm 0.03$  in the untreated site. In contrast, the proportions of virgin female moths caught in light and on interception traps placed in pheromone-treated orchards during 2000–2003 were 57 and 42% lower, respectively.

A variety of factors could affect these differences in the mating status of female codling moths observed over time, including the relative effectiveness of sex pheromone treatments, the use and type of supplemental sprays applied, or the development of behavioral or physiological resistance to the sex pheromone treatment. Isomate dispensers were used at the same density in all pheromone-treated orchards in both time periods; however, the Isomate-C dispenser used in 1994 was a prototype of the current Isomate-C PLUS dispenser and allowed significant degradation of codlemone to occur and had a strong decline in their emission rate over time (Knight 1995, Millar 1995). Nevertheless, levels of female moth virginity for moths caught in light traps were actually higher in 1994 than in 2003.

Sublethal effects from adult moth exposure to some insecticide residues can affect female moth mating success by disrupting mate location or courtship behaviors (Haynes 1988). Studies have shown that a few specific insecticides can impact the mating success of codling moth (Abivardi et al. 1998, Hoelscher and Barrett 2003, Knight and Flexner 2006); however, the potential influences of the majority of pesticides used in orchards have not been examined. Pesticide applications in the orchards included in this comparison varied widely including commercial orchards (Yakima Valley 1994 and Brewster 2000–2002), organic (Moxee 1995 and Moxee 2000–2003), and experimental research farms (Parker and Zillah 2000–2002). Data are not available to assess the potential impact of any of these supplemental spray program on the mating success of female codling moth. However, the differences observed in levels of virginity seem to be more consistently related to the time period of the study than to the spray programs used in the orchards.

There is no data that suggest that the reduction in virginity observed in orchards treated with Isomate dispensers is caused by the development of behavioral or physiological resistance by codling moth. However, an interesting parallel occurred in the management of the smaller tea tortrix, *Adoxophyes honmai* (Yasuda), in Japan. The effectiveness of Isomate dispensers emitting only one component of this species four component sex pheromone blend declined after 10 yr and was restored only when dispensers emitting the full blend were used (Mochizuki et al. 2002). Similarly, Isomate-C dispensers sex pheromones are loaded with an incomplete blend of the components found in gland extracts of female codling moths (Witzgall et al. 2001) and have been used continuously in the Moxee and Brewster orchards since 1991. Future studies should

address whether selection for an altered signaling and response channel of adult codling moths has occurred (Cardé and Haynes 2004). Meanwhile, tactics that use a more complete pheromone blend for codling moth are needed to improve the performance of this technology, especially, in orchards with high moth population densities (Witzgall et al. 2001).

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